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HSP47 siRNA conjugated with cationized gelatin microspheres suppresses peritoneal fibrosis in mice

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ABSTRACT

Heat shock protein 47 (HSP47), a collagen-specific molecular chaperone, is essential for the biosynthesis and secretion of collagen and is expressed in the fibrotic peritoneum. In the present study, we evaluated the efficacy of HSP47 small interfering RNA (siRNA) to suppress the development of peritoneal fibrosis induced by chlorhexidine gluconate in mice. We initially confirmed that biodegradable cationized gelatin microspheres (CGMs) containing HSP47 siRNA could continuously release siRNA over 21 days as a result of microsphere degradation. We then determined that a single injection of CGMs incorporating HSP47 siRNA suppressed collagen expression and macrophage infiltration, thereby preventing peritoneal fibrosis. Therefore, we suggest that this controlled-release technology using HSP47 siRNA is a potential treatment for peritoneal fibrosis. Additionally, RNA interference combined with CGMs as a drug-delivery system may lead to new strategies for knocking down specific genes in vivo.

1. Introduction

Peritoneal dialysis (PD) is an established alternative therapy for patients with end-stage renal disease. However, the peritoneal membrane undergoes various functional and structural changes over time in patients on dialysis. Loss of peritoneal function due to reduced ultrafiltration and increased small-solute transport rate is a leading cause of treatment failure in PD patients [1]. These alterations in peritoneal function are caused by structural changes in the peritoneal membrane. Long-term PD therapy leads to characteristic pathological features such as loss of mesothelial cells, marked peritoneal fibrosis and massive accumulation of collagen in the peritoneum [2–4]. The molecular mechanisms involved in the initiation and progression of peritoneal fibrosis remains unclear, and effective prevention of and treatment for peritoneal fibrosis are not established.

Heat shock protein 47 (HSP47), a collagen-specific molecular chaperone, is essential for biosynthesis and secretion of collagen molecules [5–7]. We have previously shown that HSP47 expression is markedly increased in fibrotic peritoneal tissue from patients on continuous ambulatory peritoneal dialysis [8]. Additionally, we found increased HSP47 expression in rats with chlorhexidine gluconate (CG)-induced peritoneal fibrosis [9]. We have also demonstrated suppressed peritoneal fibrosis in CG rats using antisense oligonucleotides against HSP47 mRNA [10]. These findings suggest that HSP47 plays an important role in the progression of peritoneal fibrosis.

RNA interference (RNAi) by small interfering RNA (siRNA) is an effective tool for silencing gene expression. Their gene-silencing effect is more specific and powerful than either antisense oligonucleotides [11] or dominant negative mutant genes. Although several feasibility studies have been conducted on the therapeutic efficacy of siRNA using animal models for various diseases [12–16], obstacles to siRNA use remain, such as its short period of gene-silencing effect and its low transfection efficiency. Several viral and non-viral vectors have so far been explored for the enhancement of gene transfection efficiency. Non-viral vectors have several advantages over viral vectors, such as low toxicity and immune responses and lack of integration into the genome. Gelatin has been extensively used for industrial, pharmaceutical and medical applications, and its biosafety has been proved through its long-standing clinical use as a surgical biomaterial and drug ingredient [17]. Another advantage of gelatin is that it is easy to modify. Furthermore,

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